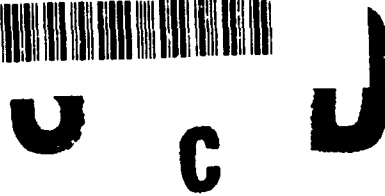


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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR EVALUATING A SIMULANT FOR TOXIC GASES IN PRIMATES

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FINAL REPORT FOR THE PERIOD JULY 1989 THROUGH AUGUST 1990

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TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-90-85

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

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PREFACE

This is one of a series of reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, NSI Technology Services. This document serves as a final report on development of a physiologically based pharmacokinetic model for evaluating a simulant for toxic gases in primates. The research described in this report began in July 1989 and was completed in August 1990 under U.S. Air Force Contract No. F33615-85-C-0532. Lt Col Michael B. Ballinger and Maj James N. McDougal served at various times as Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, during the conduct of this research. Capt Bruce Jarnot, USAF, served as coordinator of this study.

The animals used in this study were handled and maintained in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

BW	Body weight
CPFB	Chloropentafluorobenzene
FRC	Functional residual capacity
GC	Gas chromatograph
PBPK	Physiologically based pharmacokinetic

SECTION 1

INTRODUCTION

In response to the sudden threat of exposure to a toxic gas, an endangered individual can use protective equipment, such as a gas mask. To assess the effectiveness of the defensive response made by a threatened individual, an innocuous uptake simulant of the toxicant could be used in exercises that mimic the exposure scenario. The dose received by a subject during the exercise would be inferred by measuring the concentration of toxicant in expired breath after the exercise. A physiologically based pharmacokinetic (PBPK) model would provide the relationship between measurement and dose. Thereby, the efficacy with which subjects use protective gear could be evaluated and improved.

Potential simulants have been evaluated by Jepson et al. (1985) on the basis of physical properties, detectability, biological inertness, and absence of toxicity. Chloropentafluorobenzene (CPF_B), a halogenated aromatic, was identified as a suitable simulant for volatile toxicants. Inhalation exposures conducted on rats showed no toxicity (4 h, 5 mg/L) (Kinkead et al., 1987).

A PBPK model for a volatile compound in rats (Ramsey and Andersen, 1984) was modified to form a model for CPF_B in rats (Vinegar et al., 1990). Because a simulant will ultimately be used in human exposures, a PBPK model for CPF_B kinetics in primates is needed. We have developed such a model and tested it with rhesus monkey exposures. One use for the human model is to evaluate the suitability of the proposed simulant before conducting human exposures. Simulations would clarify the relationship between exposure and subsequent CPF_B concentration in expired breath. Perhaps the delivered dose would have to be impractically high, or the interval between exposure and measurement would have to be impractically brief for detection. Given the sensitivity of measurement apparatus, our simulations show the feasibility of using CPF_B as an uptake simulant.

SECTION 2

MATERIALS AND METHODS

2.1 ANIMALS

Eight rhesus monkeys (*Macaca mulatta*) were chosen randomly from a pool of 15 that had been selected from the colony maintained by the Veterinary Sciences facility at Wright-Patterson Air Force Base, Dayton, OH. Selected animals were healthy males, lacked canine teeth, and had not been subjects in another study for at least six months. Among the eight subjects, the range of ages was 6.66 years to 9.16 years, and the range of weights was 7.4 to 10.0 kg. They were maintained on a 12 h light/dark cycle in temperature- and humidity-controlled rooms (22 °C, 54%). They were provided Purina monkey chow twice a day and water *ad libitum*. Each subject was fasted 12 h prior to an exposure. Feeding was resumed upon complete recovery from anesthesia.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication 86-23, 1985, and the Animal Welfare Act of 1966, as amended.

2.2 VAPOR GENERATION AND DELIVERY TO ANIMAL

From 3 to 18 h prior to an experiment, a 300-ppm vapor of CPFB (Chemical Abstracts Service #344-07-0, Aldrich Chemical Company, Inc., Milwaukee, WI) was prepared in a 200-L Tedlar gas sample bag (SKC, Inc., Eighty Four, PA). No contaminants were detectable by gas chromatography (GC)-mass spectrometry. An exposure began when this bag was attached to the apparatus (Figure 1). A nonrebreathing valve (aluminum body; silicon rubber one-way valves, part #2608, Hans Rudolph, Inc., Kansas City, MO) was joined by two stainless steel tubes, 1.5 ft length and 5/8 in. diameter, to the source and exhaust gas sample bags. The anesthetized animal breathed through an endotracheal tube connected to the mouth port of the nonrebreathing valve.

2.3 MEASUREMENT OF CPFB CONCENTRATION

Concentration of CPFB in expired air was determined with a GC (Hewlett-Packard, Palo Alto, CA, Model HP 5890) equipped with pairs of sampling valves, 20 in. x 1/8 in. 2% type OV-101 packed columns, and flame ionization detectors. Nitrogen was the carrier gas (20 mL/min). Oven temperature was a constant 103 °C. A vacuum pump attached to the outlet ports of the sampling valves pulled gas through 1-mL sample loops (one for each valve). The sampling site was located immediately downstream from the nonrebreathing valve. The sampling valves were alternately trig-

gered so that nine samples were taken at 12-sec intervals, measured concentrations were printed out for 30 sec, and then the cycle was repeated.

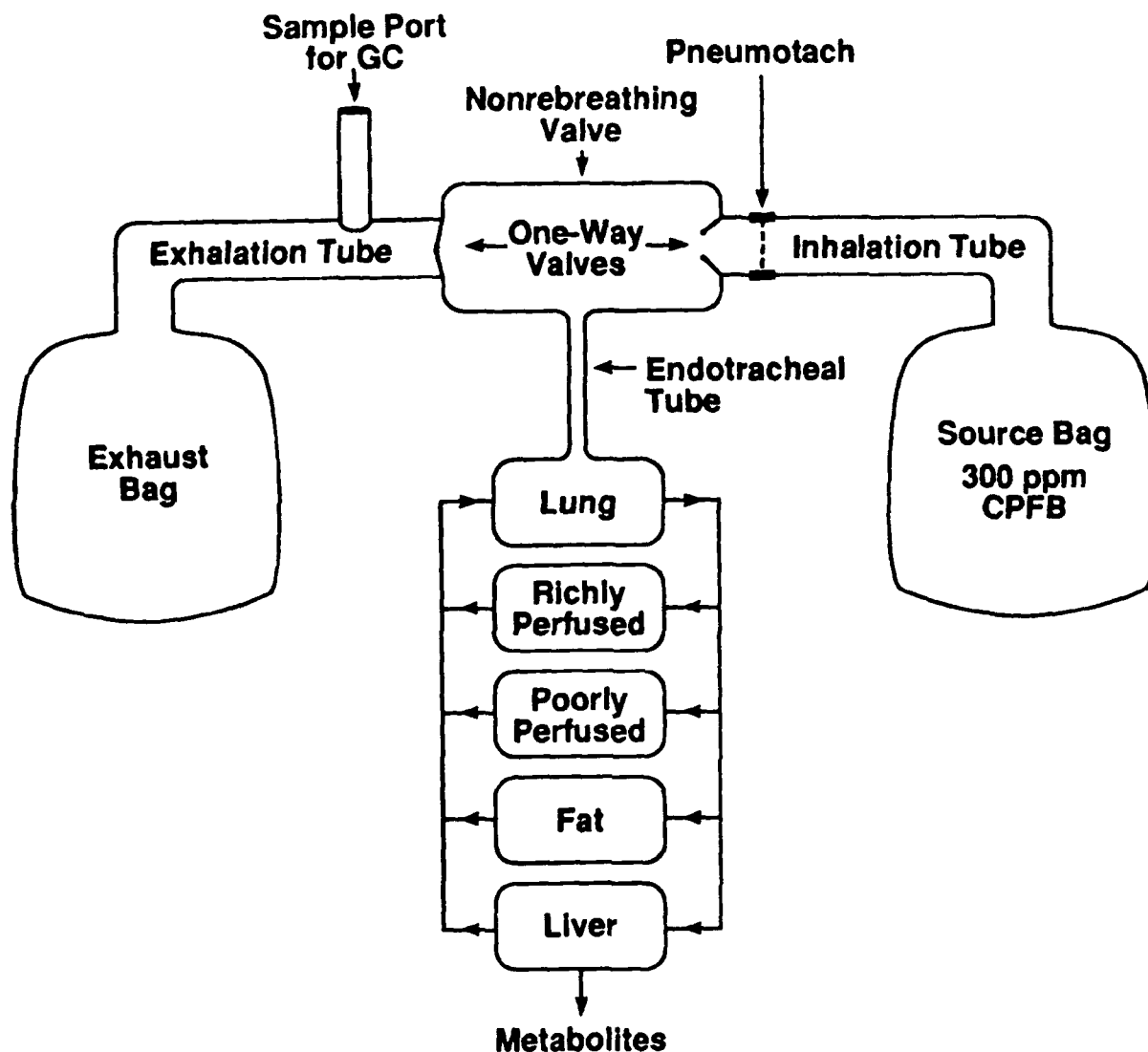


Figure 1. Schematic of the exposure apparatus and the subject. The animal is represented as a PBPK model having a bellows lung. The one-way valves are shown in the inspiratory state. During expiration, the one-way valves reverse their open and shut states.

2.4 MEASUREMENT OF VENTILATION

The flow resistance element of a pneumotachometer (model 3813, Hans Rudolph, Inc., Kansas City, MO) was located between the nonbreathing valve and the stainless steel tube on the inspiratory side. A pressure transducer (model DP 45-16, Validyne Engineering Corp., Northridge, CA) produced an electrical signal from the pressure difference across the resistance. The electrical signal was conditioned by a carrier amplifier (model 8805B, Hewlett-Packard, Palo Alto, CA), and recorded

by a digital acquisition analysis and archive system (Po-Ne-Mah Inc., Storrs, CT). Inspiratory volume, inspiratory duration, whole-breath duration, and time of inspiration initiation were recorded for each breath during an experiment.

2.5 EXPERIMENTAL PROCEDURE

Before an exposure, the GC was calibrated with the gas sample bag prepared for that exposure. As a test for linear response, a 150-ppm bag and room air were also used. The pneumotach was calibrated with a gas-tight syringe connected to the mouth port of the nonbreathing valve. A veterinarian anesthetized the monkey with Ketamine-HCl (about 17 mg/kg) and inserted an endotracheal tube. With the anesthetized monkey in the setup, a 15-min exposure began with attachment of the 300-ppm bag to the apparatus. A 15-min period of clearance began when the bag was detached.

2.6 RHESUS MONKEY MODEL

A PBPK model for CPF in rats (Vinegar et al., 1990) was modified to form the PBPK model for CPF in the rhesus monkey. Fractional sizes of richly perfused, poorly perfused, fat, and liver compartments in the rhesus monkey model were 0.09, 0.72, 0.05, and 0.05, respectively; the fractional distribution of cardiac output to the same respective compartments was 0.25, 0.53, 0.02, and 0.20 (derived from Forsyth et al., 1968; 1971). The lungs were represented as a well-mixed bellows that inflated and deflated during breathing (Appendix A). The functional residual capacity (FRC) was obtained by proportional scaling according to body weight (BW) from smaller rhesus monkeys (BW = 2.5 kg, FRC = .087 L) (Crosfill and Widdicombe, 1961). Dead space (V_D) was similarly scaled from smaller rhesus monkeys (BW = 5.3 kg, V_D = .0126 L) (Bourne, 1975). The model used tidal volume and timing of breaths recorded during an exposure to simulate that exposure. Based on the performance of rhesus monkeys under pentobarbital anesthesia (Hayden, 1964), cardiac output was set to one half ventilation. Cardiac output tracked minute-by-minute fluctuations in ventilation. Partition coefficients of rhesus monkey tissues were determined *in vitro* with a vial equilibration technique (Sato and Nakajima, 1979; Gargas et al., 1989). Tissue/blood partition coefficients for the rapidly perfused, poorly perfused, fat, and liver compartments were 3.5, 2.1, 104.1, and 8.0, respectively. The blood/air partition coefficient was 7.0. For the primate model, the first order metabolism rate constant, K_f (h^{-1}), was obtained by scaling according to $K_f = K_{fc} \cdot BW^{-0.3}$ (Gargas and Andersen, 1988). $K_{fc} = 2.0$ ($h^{-1} \text{ kg}^{-1}$) is the first order rate constant in rats, normalized to a 1-kg animal (Jepson, 1985). Because rat metabolism was observed to be a first order process, primate metabolism was assumed to be a first order process also.

2.7 APPARATUS MODEL

It was observed that the exposure apparatus affected CPFB concentration in expired air. There was absorption of CPFB by the apparatus and mixing of gases within the spaces of the apparatus. To simulate the concentration measurements during rhesus monkey exposures, an apparatus model (Appendix B) was linked to the animal model. Control experiments in which the animal was replaced with a nonabsorbing glass syringe were simulated with the apparatus part of the model. These simulations were optimized to obtain parameter values (Appendix B) for an accurate representation of the equipment.

2.8 HUMAN MODEL

The human version of the primate model also had a bellows lung and tissue compartments perfused by parallel blood flows. The fractional sizes of the rapidly perfused, poorly perfused, fat, and liver compartments were 0.064, 0.635, 0.195, and 0.026, respectively; the fractional distribution of cardiac output to the same respective compartments was 0.53, 0.14, 0.09, and 0.24 (Reitz et al., 1987). Tidal volume was 0.5 L; FRC was 2.3 L; V_D was 0.15 L; and respiratory rate was 12 breaths per min (Guyton, 1986). Rhesus monkey partition coefficients were used. The ratio of cardiac output to ventilation was assumed to be one.

Using the human model (without apparatus), brief exposures (1 to 10 breaths) followed by a long clearance period (12 h) were simulated. A brief exposure was used to simulate the conditions that might arise due to improper or delayed donning of a chemical defense respirator during training. Simulations were conducted for 12 h to determine the persistence of the simulant after exposure.

Models were written in the FORTRAN computer language and run on a VAX 8530 (Digital Equipment Corp., Maynard, MA).

SECTION 3

RESULTS

CPFB concentration measurements from a typical experiment and its simulation are shown in Figure 2. During exposure, absorption of CPFB by the animal reduced concentration from 300 ppm in inspired air to about 150 ppm in expired breath. Following exposure, the CPFB that cleared from the animal appeared in expired breath. A similar plot was generated for each monkey exposure (not shown). From each of these plots, one panel of Figure 3 was derived.

The points from Figure 2 were grouped (nine measurements to a group; nine simulations to a group) and averaged to produce Figure 3B. Measurements are shown as mean \pm standard deviation; simulations are shown as a continuous line. There is a good match between the simulations and experimental measurements from seven of eight animals (Figure 3).

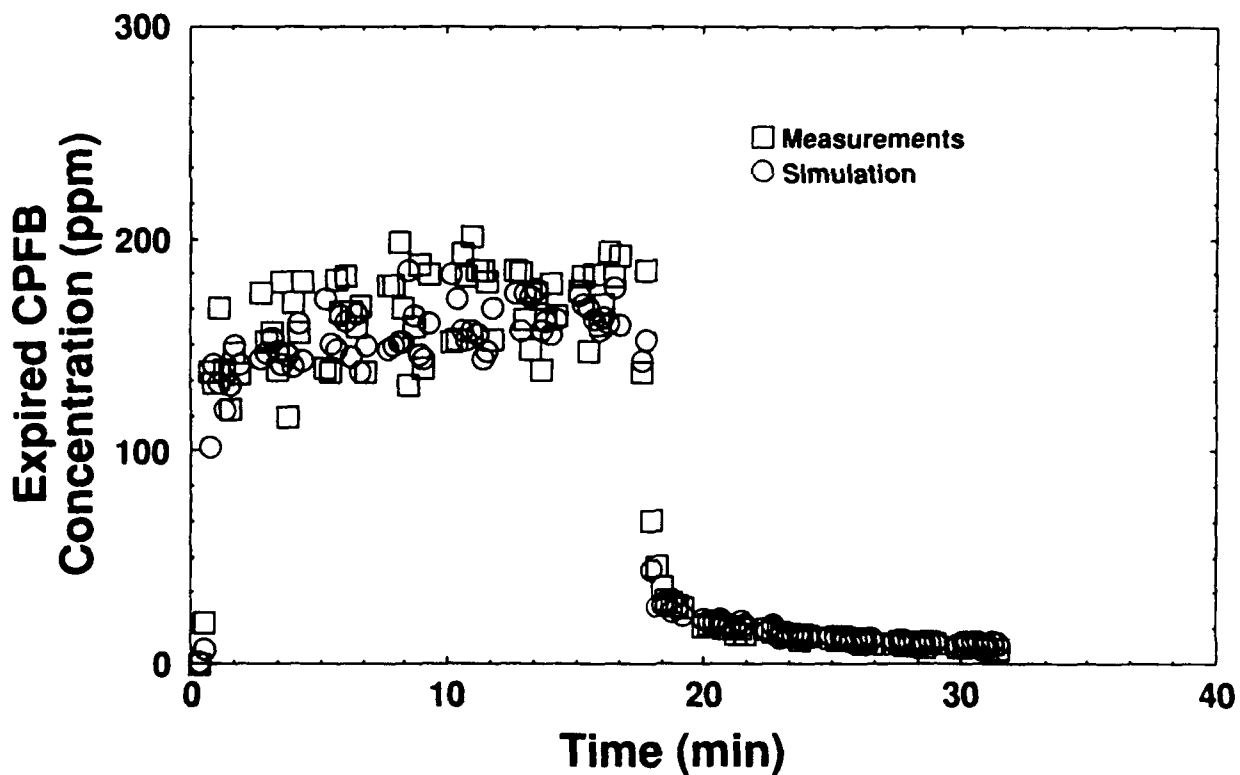


Figure 2. CPFB concentration in expired breath during one experiment. (□) Measured concentration; (O) Simulated concentration. During exposure the concentrations are about 150 ppm; during subsequent clearance, about 10 ppm. Concentration in the gas sample bag used for exposure was 300 ppm.

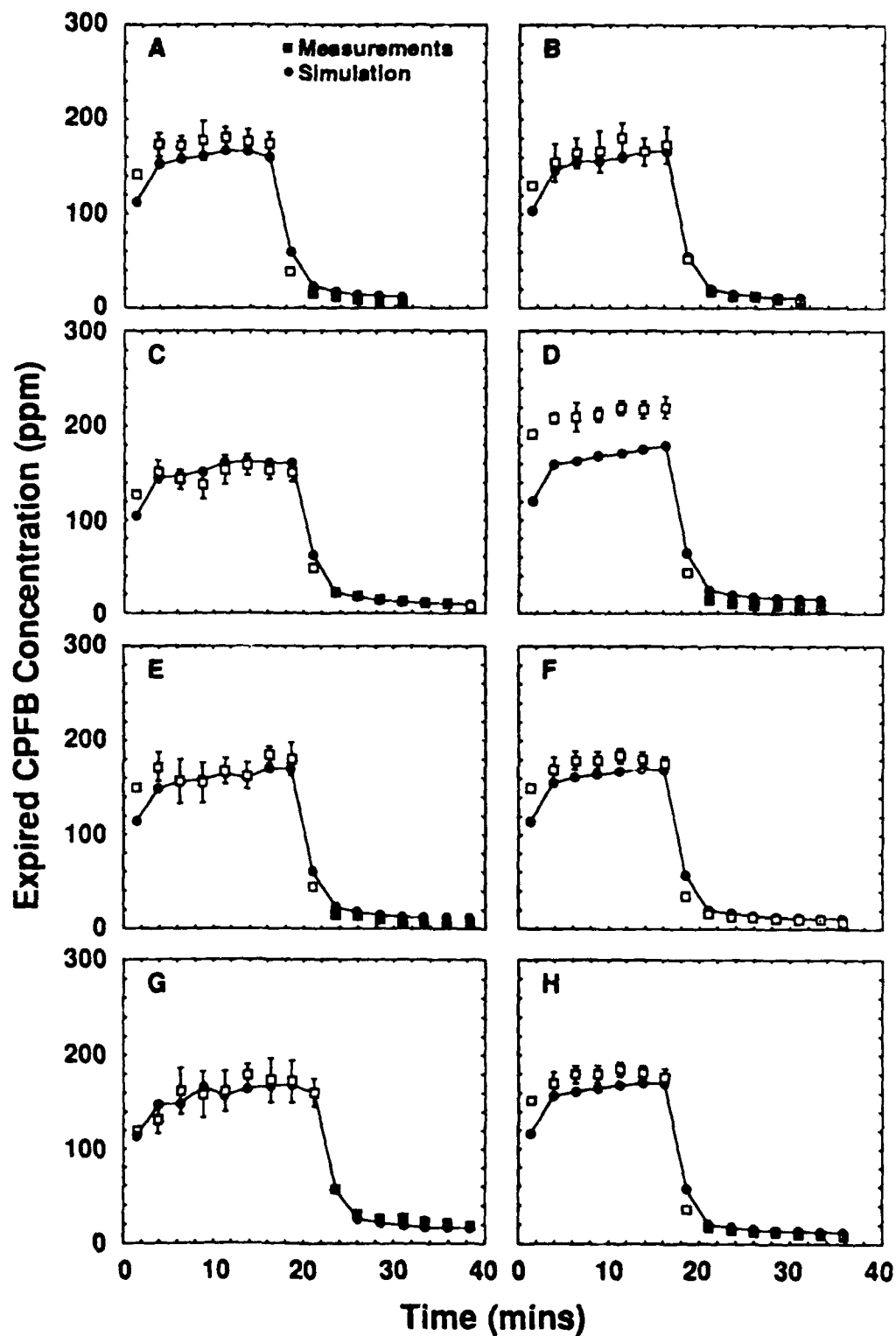


Figure 3. CPFB concentration in expired breath during eight experiments. Figure 3B was derived from Figure 2. Other panels were similarly derived. (\square) Mean \pm SD from nine consecutive measurements; (\bullet) Mean from nine consecutive simulated concentrations, connected to produce a continuous curve.

Using the human model, a brief inhalation exposure to CPF_B was simulated. The concentration in end-expired breath for the postexposure interval, ranging from 10 min to 12 h, was plotted (Figure 4). The dose was varied by changing CPF_B concentration in a single-breath exposure (Figure 4A), and the number of breaths containing a CPF_B vapor of 1 ppm concentration (Figure 4B). Increasing inhaled concentration by a factor (10 or 100) increased the clearance curve by the same factor; increasing the number of exposure breaths by a factor (3 or 10) increased the clearance curve by that factor.

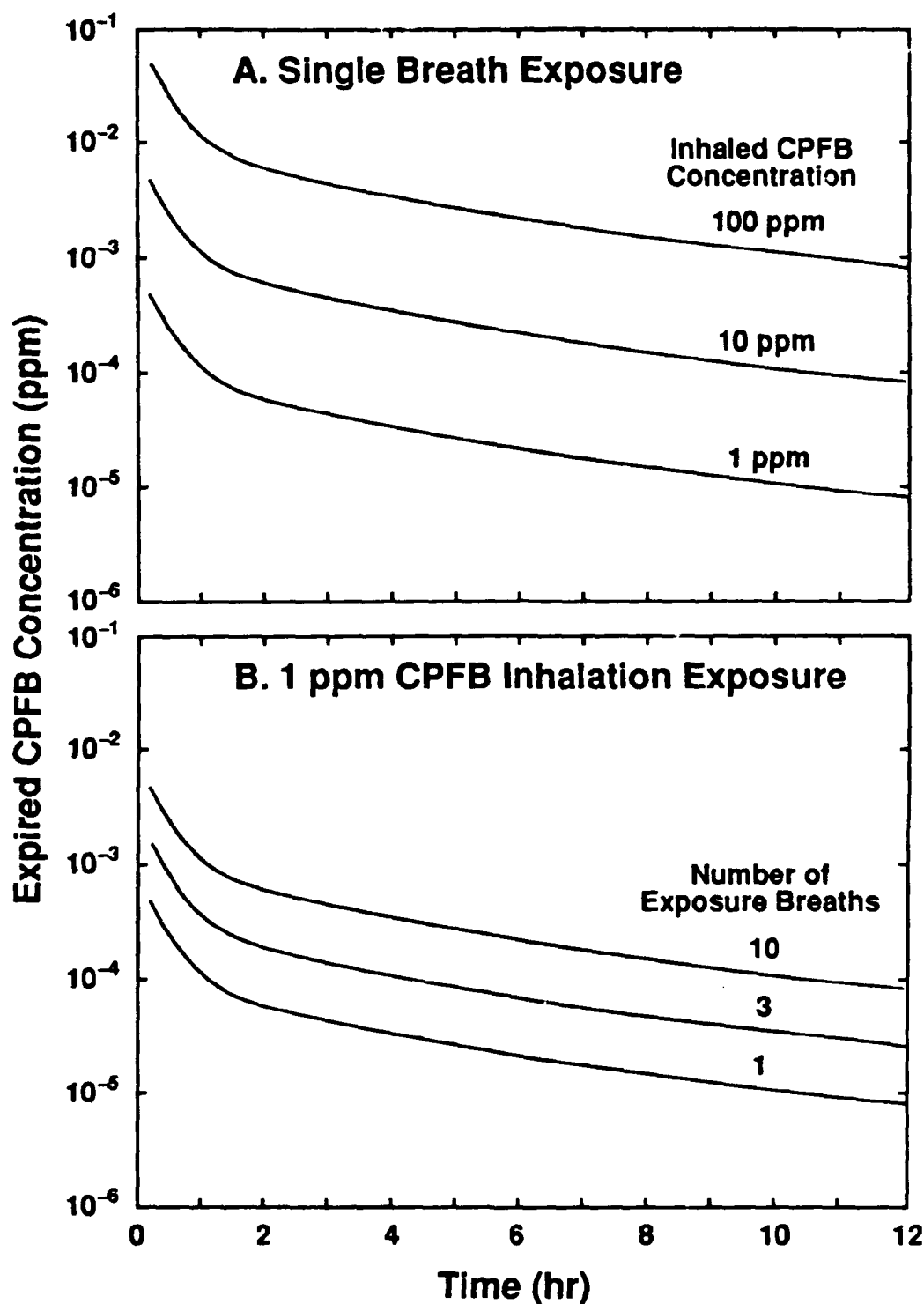


Figure 4. CPFB concentration in expired breath simulated with the human model. Plots span the interval of clearance beginning at 10 min after the end of exposure, ending at 12 h. (A) Exposure is a single breath of air containing CPFB vapor at a concentration of 1, 10, or 100 ppm. (B) Exposure is 1, 3, or 10 breaths of air containing CPFB vapor at a concentration of 1 ppm.

SECTION 4

DISCUSSION

Previous studies using CPFB with rats showed no evidence that metabolism or intercompartmental partitioning was nonlinear or saturable (Jepson et al., 1984; Vinegar et al., 1990). For this reason, and to minimize the number of exposures given to the monkeys, only one CPFB vapor concentration (300 ppm) was used.

The monkeys were lightly anesthetized, which resulted in irregular breathing patterns (Dr. J. Cooper, personal communication). There was breath-to-breath variability in tidal volume, end-expiratory lung volume, duration of the breathing cycle, and the proportion of that cycle occupied by inspiration or expiration. This variability was one cause of scatter in measurements (Figure 2). Another cause was the sampling of expired breath at regular intervals, which resulted in sampling at different phases of the breathing cycle.

Even though measured tidal volume was used for ventilation in the simulations, experimental scatter was not matched. This mismatch was probably due to breath-by-breath variation in end-expiratory lung volume, giving rise to unequal inspiratory and expiratory volumes. The simulations could not take into account these unequal volumes because only inspiratory volumes were measured.

Despite disparity between an expiratory volume and the preceding inspiratory volume, an average of the expiratory volume over several breaths must nearly equal the corresponding average inspiratory volume. Average concentrations are plotted (Figure 3) to compare simulations to measurements.

There was good agreement between simulations and measurements (Figure 3) from seven of the eight monkeys. Such agreement is lacking in Figure 3D. Although this monkey was not visibly ill during the experiment, he began to lose hair and failed to respond to external stimuli several days afterward, which suggests that he may have been abnormal at the time of the experiment. These measurements (Figure 3D) are consistent with increased intrapulmonary diffusion resistance between gas and blood. Such an increase would reduce both CPFB uptake during exposure and CPFB clearance after exposure, compared to a simulation of the experiment.

With a valid primate model for CPFB kinetics, situations that have not been amenable to experimental investigation can be simulated. The intended use of CPFB provides such a situation. In studies with humans, exposure to CPFB will be assessed afterward from a measurement made on expired breath. Simulations can address the question of whether the detection limit of the measurement apparatus will restrict the practicality of the procedure. Perhaps the delivered dose

would have to be impractically high, or the interval between exposure and measurement would have to be impractically brief.

In a simulation of a minimal exposure to CPFB (one breath; 1 ppm) followed by a prolonged clearance (12 h), the concentration of CPFB remaining in expired breath was ten parts per trillion (ppt) (Figure 4). This concentration is somewhat higher than the minimal detectable concentration (0.80 ppt) of a portable GC (Harold O. Seigel, Scintrex Ltd., Concord, Ontario, Canada, personal communication). These simulations imply suitability of CPFB as a simulant for retrospectively assessing exposure.

In a training exercise with a known simulant concentration in environmental air, an estimate of the number of exposure breaths could be made from a measurement of CPFB in expired breath, assuming the interval between exposure and measurement to be known. This would be a useful gauge of the effectiveness of protective equipment and its proper use.

SECTION 5

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APPENDIX A

To represent the lungs as a reciprocating bellows, lung volume and intrapulmonary CPFB are computed throughout the course of inspiratory and expiratory phases of breathing. Expressions to be integrated during inspiration are

$$dA_1/dt = Q_c(C_v - C_a) + C_d V_t / T_i$$

and

$$dV_1/dt = V_t / T_i$$

Expressions to be integrated during expiration are

$$dA_1/dt = Q_c(C_v - C_a) - C_1 V_t / T_e$$

and

$$dV_1/dt = V_t / T_e$$

At any instant, intrapulmonary CPFB concentration and amount are related by

$$C_1 = A_1 / V_1$$

Intrapulmonary equilibration of CPFB between blood and air is characterized by

$$C_a = C_1 P_{b,a}$$

In the above expressions A_1 is the amount of intrapulmonary CPFB; V_1 is lung volume; V_t is tidal volume; Q_c is cardiac output; C_v , C_a , C_d , and C_1 are concentrations of CPFB in venous blood, arterial blood, dead space, and the alveolar space of the lungs; T_i is duration of an inspiration; T_e is the duration of an expiration; $P_{b,a}$ is the blood/air partition coefficient.

APPENDIX B

The apparatus was modeled to account for its effect on measured CPFB concentration. At the beginning of an exposure, gases of different CPFB concentrations mixed in the (inhalation) stainless steel tube. Measurements showed the CPFB concentration profile at the animal end of the tube to be a sigmoid, as a function of ventilation. In the apparatus model that sigmoid was represented with a hyperbolic tangent

$$C_{pt} = C_s (1 + \tanh(F_m (V_{tot} - V_{tube}))) / 2.$$

C_{pt} is the concentration at the proximal end of the inhalation tube; C_s is the concentration in the source bag; F_m is a factor controlling steepness of the sigmoid; V_{tot} is total gas volume moved by ventilation; and V_{tube} is volume of the inhalation stainless steel tube (0.225 L).

Gas mixing occurs in the dead space of the nonrebreathing valve. The rate at which the amount of CPFB in that space changes depends upon the concentration of gas flowing in and out and upon absorption by the one-way valves, which are made of silicon rubber. During inspiration

$$dA_d/dt = (C_{pt} - C_d) V_t / T_i - K_r (C_{pt} - A_{v,i} / K_a)$$

$$- (K_r / 2) (C_d - A_{v,e} / K_a).$$

During expiration

$$dA_d/dt = (C_1 - C_d) V_t / T_e - (K_r / 2) (C_d - A_{v,i} / K_a).$$

A_d is the amount of CPFB in the dead space; $A_{v,i}$ and $A_{v,e}$ are the amounts of CPFB absorbed by the one-way valves on the inspiratory and expiratory sides of the apparatus, respectively; C_d and C_1 are CPFB concentrations in the dead space and the lung; The coefficient K_r characterizes the rate of absorption by a valve, and the coefficient K_a is equal to valve volume \times the valve/air partition coefficient. It has been assumed that gas flowing through an open valve exposes both sides of the valve to the same CPFB concentration.

The region sampled for concentration measurements was represented as a well-mixed volume (0.020 L) downstream from and adjacent to the expiratory one-way valve. The rate of change of the amount of CPFB in that volume during expiration was represented as

$$dA_{sam}/dt = (C_d - C_{sam}) V_t / T_e - K_r (C_d - A_{v,e} / K_a).$$

During inspiration the representation was

$$dA_{sam}/dt = (K_r / 2) (C_{sam} - A_{v,e} / K_a).$$

A_{sam} and C_{sam} are the amount and concentration of CPFB in the volume sampled by the GC.

To find the best values of F_m , K_r , and K_a , control experiments were done. The animal was replaced with a nonabsorbing glass syringe. In simulations of control experiments, the three parameters were varied to find values that resulted in the best fit of simulation to experiment.

QUALITY ASSURANCE

The study, "A Physiologically Based Pharmacokinetic Model for Evaluating a Simulant for Toxic Gases in Primates," was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of these guidelines. Phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:

April 23, 1990

April 24, 1990

August 17-20, 1990

ITEM INSPECTED:

Calibration and exposure bag preparation.

Animal exposure.

Final report.

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M. G. Schneider

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QA Coordinator

Toxic Hazards Research Unit

Date 20 Aug 90